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THE SMOOTH TO ROUGH FORM CHANGE IN BACTERIUM TYPHI., by DR. R. Th. Scholtens

Introduction

Arkwright (1921) described the smooth to rough form change. He distinguished between the smooth form, which had a smooth colony form (smooth surface and smooth edge) and was stable in salt solution, and the rough form, which consisted of a rough colony (rough surface and uneven edge) and which was auto-agglutinable. These two forms did not only differ outwardly or by culture but also immunologically. The smooth forms were virulent, the rough avirulent. Furthermore the smooth form provided vaccines which had immunizing qualities, while vaccines prepared with rough forms had no immunizing powers.

Soon afterwards, Bruce White (1925) discovered a difference in the antigen structure. He showed that the rough form lacked the thermostable antigen, which is found in the smooth form. One assumes that the immunological differences between smooth and rough forms are based on serological differences.

The rough form does not immunize, because it is lacking these antigens. Furthermore it is avirulent while the smooth form which has these antigens is virulent. One may therefore attribute a virulence importance to the antigens.

All these outside, immunological and serological differences together constitute the smooth and rough form change. The exterior particulars are far less important than the virulence. The smooth form of a type of bacteria must be virulent, while the rough form must definitely be avirulent.

However, because of practical reasons it is impossible to study the virulence of every strain. The virulence still is connected with the antigen structure. If a strain receives the thermostable antigens, on which the virulence is dependent, it is called a smooth form, it is considered a rough

form if it does not conceive these antigens. The smooth-rough form change has become a serological notion. A strain which lacks the antigens, to which virulence importance is attached, may contain a typical smooth colony form without being autoagglutinable.

Such a strain is defined "what concerns the antigen is rough, but all connected with outside characteristics is smooth".

Until a few years ago only two antigens of salmonella typhus were known, the thermostable body antigen (O-Antigen) and the thermostable flagellar antigen, the H-Antigen.

Felix (1924) discovered a third antigen, the Vi-Antigen, which is also a body antigen.

Most strains which are found during routine research work, contains both body antigens, O and Vi antigens.

Salmonella typhus has four body antigen variations.

Typhus I contains O and Vi-antigens.

Typhus II contains only O-Antigen.

Typhus III contains only Vi-Antigen.

Typhus IV contains either O or Vi-antigen.

Kauffmann calls the Vi-Antigen loss V-W-Form change.

O-Antigen loss is described as smooth-rough form change, for it is believed that strains containing no O-Antigen are always avirulent.

I have already mentioned that a certain strain may be rough serologically, while having smooth outward characteristics.

A common occurrence with Salmonella typhoid is for a strain to have no O-Antigen, while still maintaining the outward characteristics of the smooth form.

One should therefore note that these outwardly smooth characteristics are not dependent on the antigen.

However I will prove that in the case of salmonella typhus, which exhibits such great differences between the roughness, controlled by the antigen (lack of O-Antigen), and the smooth outward characteristics, a strong parallel exists between antigen structure and external characteristics.

Then however one must not only notice the O-Antigen but also the Vi-Antigen.

So -- to describe a certain antigen loss as having passed from the smooth to the rough form, one claims that this antigen loss is connected with virulence loss; one does not suggest that the appearance of exterior rough characteristics are connected with this antigen loss.

I will finally explain certain circumstantial steps for the first time.

I will set up a new plan for smooth and rough form changes based on the newly acquired data.

1. Description of Technique and Strains.

I use the adsorption method in proving the antigen structure of my strains, because numerous autoagglutinable strains must be studied.

a. Research on the presence of O-Antigen.

This occurred with Gartner serum which agglutinated the known typhus strain H 901 to a count of 1/12800 agglutinated.

1 ccm of a suspension of the strain to be studied was placed in a ccm of 1/100 serum solution. This suspension was procured from residue from 2 agar cultures of 3 ccm of 0.9% table salt solution.

This mixture was kept in a refrigerator for 24 hours.

It was then centrifuged and the remaining liquid including strain H 901 was drawn off.

The studied strain kept the O-Antigen after adsorption when the serum showed a strong titer drop, for example down to 1/800 or 1/400. The strain contained no O-Antigen if the titer remained as high as before adsorption.

b. Research on the presence of Vi-Antigen.

So as to complete the adsorption method successfully, a serum had to be prepared which contained no agglutinin other than Vi-Agglutinin effective against typhus bacteria.

This serum had an O-Titer of 1/50, an H-Titer of 1/6400 and a Vi-Titer of 1/1600. It was dissolved to a 1/50 solution with a very thick H 901 strain suspension. (The culture in a Roux tube inoculated with H 901 was suspended in 30 ccm of table salt solution.)

It was centrifuged after remaining in the refrigerator for one hour. The remaining liquid no longer agglutinated the H strain and contained no more H or O-agglutinin.

The correct adsorption test was conducted with this remaining liquid. A ccm of this liquid was added to 1 ccm of the strain suspension to be tested. This mixture was centrifuged after a 24 hour refrigeration period and was titrated with the strain 4516. If this strain did not agglutinate at all, or did very lightly, it contained Vi-Antigen.

If the titer had remained high the tested strain would have contained no Vi-Antigen.

The results of the tested strains of every typhus were as follows:

50 strains containing O or Vi-Antigen, were isolated in the bacteriological

section from our research material.

Strains containing only O-Antigen, could be found in our collection, and furthermore I had the Ty 8 and H 901 strains, which Dr. Felix had sent me with a number of other strains.

I also received strains containing Vi-Antigen, but no or little O-Antigen, from Dr. Felix (6 S Ty 441 II, Giglioli Rb I).

Strains Ty 4516 and RU were isolated in the diagnostic section.

Three other strains of this antigen structure were obtained from strains, containing O and Vi-Antigen, which had been transferred through broth with added Gartner serum. I will go into further detail in Section 4.

A last strain was obtained from an old broth culture.

I obtained strains containing either O or Vi-Antigen from Dr. Felix (Ty 2 R 20b, Ty Mrs. S. r 2b. M.U., Ty 8. Al. RI) and from Prof. Snyders in Amsterdam.

I further isolated 9 of these strains by transferring strains containing only O-Antigen or O and Vi-Antigen through broth with Gartner serum.

2. Connection Between Smooth Characteristics and Vi-Antigen.

The antigen structure of the strains described in the last section was proven by the method given therein. Their smooth or rough characteristics were observed simultaneously.

It is a known fact that strains containing O and Vi-Antigen, and strains which contain O-Antigen as the only body antigen, have smooth outward characteristics. My 50 strains containing O and Vi-Antigen, and the 9 strains with only O-Antigen, had a smooth colony form and were stable in table salt solution

(0.9% NaCl). It is also known that typhus strains, which contain no O-Antigen (and which therefore are rough forms according to Felix (1935), Kauffmann (1936) and others) react differently with respect to smooth characteristics.

They sometimes are rough forms outwardly, but at times they may be stable in salt solution and even have a smooth colony form. Such strains without O-Antigen with outward smooth characteristics are described by Kauffmann, Felix and others, as: "rough in what concerns antigen, but having smooth outward characteristics".

It happened that 21 of the strains studied by me had no O-Antigen.

8 of these strains had outward smooth characteristics.

3 of these 8 strains were stable in salt solution and had a smooth colony form.

The 5 remaining had as only outward smooth characteristic stability in table salt solution, but had rough colony forms.

The 13 remaining strains were autoagglutinable and had rough colony forms.

When I tested these strains for Vi-Antigen content by the method described in Section 1, I discovered that all 8 strains which had outward smooth characteristics contained Vi-Antigen. However, the 13 strains which had no outward smooth characteristics contained no Vi-Antigen.

The presence of Vi-Antigen in strains which contain no O-Antigen, is always connected with the presence of outward smooth characteristics, i.e., always with stability in table salt solution and sometimes also with smooth colony forms.

A strain is autoagglutinable and has a rough colony form, whether it contains O or Vi-Antigen.

3. The Influx of Vi-Bacteriophages on Strains Containing Vi-Antigens and No O-Antigens.

The connection between Vi-antigen and outwardly smooth characteristics appeared clearly, when straining forms which do not contain Vi-antigen, from strains which have Vi-antigen as the only body antigen.

This may easily be achieved with so called Vi-Bacteriophages.

Vi-Bacteriophages are typhoid bacteriophages which show an affinity for Vi-Antigen.

It only attacks typhus strains which contain Vi-Antigen. However, a second fact is more important in this connection. The secondary cultures remaining after penetration of bacteriophages contain no Vi-Antigen.

The scholars (Craigie and Brandon, 1936; Sertic and Boulgakov, 1936; Scholtens, 1936) who have described these characteristics did research only on the secondary cultures of strains containing O and Vi-Antigens.

These secondary cultures contained O-Antigen as the only body antigen and had outward smooth characteristics.

I tested all strains described in Section 1 for susceptibility to Vi-Bacteriophages by placing a drop of bacteriophage into an agar tube inoculated with the corresponding strain.

All strains containing O and Vi-Antigen or only Vi-Antigen were susceptible to this bacteriophage.

The secondary cultures resulting from the addition of these bacteriophages were then studied for antigen structure and external smooth and rough characteristics. Two bouillon tubes were inoculated for this purpose. A few drops of bacteriophages were added to one of these tubes.

The secondary culture appeared after a slow growing period at the start. This was placed on agar plates and various colonies were inoculated from it

and studied further.

The secondary cultures of the 50 strains which had O and Vi-Antigen only kept the O-Antigen and had smooth outward characteristics.

The 8 strains which contained Vi-Antigen but no O-Antigen were studied in the same manner.

The secondary culture, which appeared after lysis of these strains by Vi-Bacteriophage showed a fluffy growth.

The colony contained the following when placed on agar plates. Only rough colonies appeared, except in cases where the original strains had had a smooth colony form, in which case a few smooth colonies appeared on the plate between numerous rough colonies.

These smooth colonies when re-innoculated produced colonies which also resembled the original strain in other ways.

They were still susceptible to Vi-Bacteriophages, contained Vi-Antigen and were not autoagglutinable.

The rough colonies produced cultures through inoculation, which were not susceptible to bacteriophages, had lost the Vi-Antigen and were auto-agglutinable.

These strains had lost their outward smooth characteristics with the Vi-Antigen through influence of Vi-Bacteriophages.

These strains received no outward smooth characteristics because of the influence of other bacteriophages, which had no tendency toward Vi-Antigen.

All this indicates the connection between Vi-Antigen and outward smooth characteristics.

4. Connection Between O-Antigen and Outward Smooth Characteristics.

Outward smooth characteristics are always connected with presence of O-Antigen. Strains containing O and Vi-Antigen, or strains which contain O-Antigen as the only body antigen are autoagglutinable.

With this exception, strains which have this serological structure usually contain a typical smooth colony form.

It is a known fact that typhus strains may loose the O-Antigen when strained in bouillon, with added Gartner serum. (Through growth by presence of the homologic antibody.)

I did research on the presently entering changes of outward smooth forms.

15 typhus strains, of which 8 contained both O and Vi-Antigen and 7 only O-Antigen, were inoculated in 10 ccm bouillon, to which had been added 0.1 ccm of Gartner serum. This Gartner serum had an O-titer of 1/12800 and contained 1/2% of carbolic acid as a preservative. The concentration of the latter in the nutritive base was naturally very low.

These strains were inoculated into fresh bouillon with Gartner serum after a week. It was placed on an agar plate after 2 such transfers and various colonies were inoculated. The obtained cultures were studied for O and Vi-Antigen.

Thus from 6 out of 7 strains which only had O-Antigen, subcultures were isolated which no longer had this antigen. They always were agglutinable and had a rough colony form.

A strain which possesses O-Antigen as the only body antigen becomes autoagglutinable when this is lost. The colony form becomes typically rough. Among the 8 strains, which contain O and Vi-Antigen one was able to separate 6 strains, which no longer had O-Antigen. All showed a rough colony form.

Furthermore 3 were autoagglutinable.

It appeared that the latter had not only lost the O-Antigen through transferal, but also the Vi-Antigen. The 3 other subcultures were not agglutinable.

They no longer contained O-Antigen but had Vi-Antigen.

A strain which contains O- and Vi-Antigen and only loses the O-Antigen does not become autoagglutinable. However, it becomes autoagglutinable if it also loses the Vi-Antigen.

One may state the following in conclusion:

If a strain contains either O-Antigen or Vi-Antigen, it had outward smooth characteristics (always stable in salt solution, often also a smooth colony form). The more so if it contains both antigens.

It is nonsense to assume that factors exist which may give outward smooth characteristics to a strain, devoid of both of these antigens.

5. Why strains, Containing O and Vi-Antigen Receive no Outward Rough Characteristics Under the Influence of Vi-Bacteriophages, While Strains Which Have Vi-Antigen as the Only Body Antigen, do.

Both O and Vi-Antigens are factors which may give a strain outward smooth characteristics. A strain, containing O and Vi-Antigens, therefore has two factors, both of which may give it outward smooth characteristics.

Such a strain loses one of these factors in Vi-Antigen. The presence of the other (the O-Antigen) however is enough to maintain its outward smooth characteristics.

A strain which contains Vi-Antigen as the only body antigen, under the influence of Vi-Bacteriophages loses the only outwardly smooth characteristics' building factor, when it loses the Vi-Antigen.

Therefore, these strains receive outwardly rough characteristics when influenced by Vi-Bacteriophages.

6. On the Influence of Vi-Antigen on Virulence.

While an antigen loss, connected with virulence loss, is known as a smooth-rough form change, Vi-antigen loss, of immediately recognized significance to virulence, is not called smooth-rough form change, but V-N-Form change.

The basis for this is that Felix ascribed Vi-Antigen a special role in connection with virulence, analogous to a manifestation in vitro.

When a strain contains Vi-Antigen besides the O-Antigen, one may notice that it absorbs the O-Agglutinin but is hardly or not at all agglutinated by the absorbed antibody.

Vi-Antigen in vitro protects O-Antigen against the intrusion of O-Antibodies. This protective action would increase in vitro and therefore raise the virulence through the O-Antigen.

Vi-Antigen could have no influence on virulence when not in the presence of O-Antigen.

Strains of the Type III, which contains Vi-Antigens, but no O-Antigen, are avirulent after his experiment.

Felix isolated a number of such strains which I have also used in my work (6 S Ty 441 II) from old bouillon cultures.

These were avirulent. Only a subculture of the Giglioli strain, which had Vi-Antigen and a small quantity of O-Antigen showed signs of virulence. He ascribed the virulence to the remaining O-Antigen contained in this strain.

A strain studied by Pot (1936), Ty 4516, was virulent without containing

any O-Antigen.

250 million germs of this type killed 5 out of 5 mice, when injected intraperitoneally.

No O-titer higher than 1/50 was found in injected rabbits. It became agglutinable when influenced by Vi-Bacteriophages. It absorbed no O-Agglutinin.

It is impossible to ascribe the virulence of this strain described by Pot to the O-Antigen. It is probable that the Vi-Antigen may add virulence to certain strains even in the absence of O-antigen.

The strains studied by Felix, which contained Vi-Antigen, but no O-Antigen were avirulent. This may be explained by the fact that these contained Vi-Antigen in smaller quantities. This need not be a coincidence, it may be caused through long straining in bouillon.

On the other hand strain 4516 was a freshly insulated strain. The further virulence growth caused by the Vi-Antigen is independent from O-Antigen. Naturally this does not mean that a strain having O and Vi-Antigen is not more virulent than a strain containing only Vi- or only O-Antigen in the same proportion and does not exclude a potential activity of both antigens.

7. Why Vi-Antigen Has a Part in Smooth-Rough Form Change Which is to Balance That of O-Antigen.

I stated in the introduction that with typhus bacillae one may prove, not along virulence loss, but also loss of outward smooth characteristics in connection with antigen loss, which will be called "smooth-rough form change".

That a virulence loss is connected with the loss of Vi-Antigen is a known fact. However not only virulence loss, but loss of outward smooth characteristics is connected with this antigen loss. The fact that this only showed with strains containing O-Antigen has been cleared up.

All requirements to call the Vi-Antigen loss a "smooth rough form change" are therefore fulfilled.

Virulence loss is also connected with O-Antigen loss (as generally accepted). A loss of outward smooth characteristics is connected with the loss of O-Antigen in strains devoid of Vi-Antigen. O-Antigen and Vi-Antigen therefore fulfill the same conditions. A plan describing these relations will be set up in the next section.

8. New Plan for the Smooth-Rough Form Change of Bacterium Typhus.

The following nomenclature considers the natural relationships.

I distinguish:

- I. Smooth form, contains O and Vi-Antigen.
- II. O-Semismooth form, contains O-Antigen.
- III. VI-Semismooth form, contains Vi-Antigen.
- IV. Rough form, contains either O or Vi-Antigen.

These four forms may contain H-Antigen.

The smooth form contains all the attributes of this form in the plan: highest virulence count, smooth colony form, stability in table salt solution.

The rough form contains all the characteristics of its form, such as avirulence, rough colony forms, and autoagglutinability.

The forms, which contain only one of the two body antigens, have serologically half changed from smooth form to rough form.

This is why I call them half smooth forms.

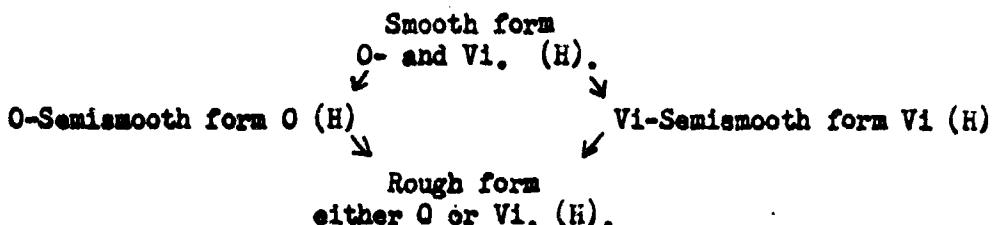
These forms are middle of the way forms, even in other characteristics.

The O-Semismooth form has lost a part of its virulence. The same hold true for the Vi-Semismooth form. (As generally accepted virulence loss, is con-

nected with loss of O-Antigen.)

Moreover, the Vi-Semismooth form usually has a rough colony form. However agglutinability appears only during the transition from semismooth forms to rough forms. The strain also receives all other rough characteristics, which it did not have before.

The following diagram again gives the smooth-rough form change.



9. Comparison of My Plan to Kauffmann's.

When comparing Kauffmann's plan to mine, one may notice that the same strains are brought together in one type.

Kauffmann distinguishes:

- I. V-W-Form change, variations in Vi-Antigen (the V-Form contains Vi-Antigen while the other does not).
- II. Smooth-rough form change, variations in O-Antigen (the smooth form contains O-Antigen, the rough does not).

In V-W-Form change he has studied all strains which contain O-Antigen; he hardly mentions the V-W-Form change of strains which contain no O-Antigen.

I have exhaustively proved that Kauffmann's V-W-Form change is a smooth-rough form change.

Kauffmann's V-(Smooth)-Form equals my smooth form (Vi- and O-Antigen).

Kauffmann's W-(Smooth)-Form equals my O-Semismooth form (O-Antigen).

Kauffmann's V-(Rough)-Form equals my Vi-Semismooth form (Vi-Antigen).

Kauffmann's W-(Rough)-Form equals my rough form (either O or Vi-Antigen).

10. Vi-Bacteriophages as diagnosticphages.

Sonnenschein used bacteriophages for diagnostic purposes. He used typhus-specific bacteriophages for typhus diagnosis with results.

It is known today that the presance of Vi-Antigen is a necessary condition for the typhus strain sensitivity of these bacteriophages.

As most typhus strains discovered during routine examinations contain Vi-Antigen, the Vi-Bacteriophage may be used as diagnosis bacteriophage.

Craigie (1936) described how he diagnosed Kauffmann's V-Form with the Vi-Bacteriophage. However one cannot distinguish between strains containing Vi and O-Antigen or only Vi-Antigen, when using Vi-Bacteriophages.

However, the four forms described above are distinguished as follows, if one observes the autoagglutinability of the strain, and the secondary culture after lysis with the Vi-Bacteriophages.

Form I (O and Vi) and Form III (Vi) are both sensitive to Vi-Bacteriophages. The Form I secondary culture is not autoagglutinable as opposed to that of Form III.

Form II and Form IV are both unsensitive to Vi-Bacteriophages.

However, one may distinguish them because Form IV is autoagglutinable while Form II is not.

Summary

Although smooth-rough form change is a serological concept, one may not call every antigen loss a smooth-rough form change.

A virulence loss must be connected with this antigen loss.

One may state that a loss of outward smooth characteristics is connected with this antigen loss in the typhus bacterium.

Not only O-Antigen but also Vi-Antigen suffices for this demand.

The reasons for which outward rough characteristics only arise in certain cases of loss of these antigens have been explained.

The important fact, that not only O-Antigen loss, but also loss of Vi-Antigen constitutes a smooth-rough form change, has created a new plan for the smooth-rough form change of Bacterium typhi.

In the second place, a method has been described, with which by using Vi-Bacteriophages one may identify all variations of Bacterium typhi body antigens.

Conclusions

I. There exists parallelity between outward smooth characteristics and antigen structure of Bacterium typhi.

II. Strains which contain either O or Vi-antigens, and therefore more strains containing both O and Vi-Antigen, are not autoagglutinable and usually have a smooth colony form.

III. Strains which have O-Antigen or O and Vi-Antigen are autoagglutinable and have a rough colony form.

IV. No factors may create outwardly smooth characteristics in typhus bacteria, in the absence of these two antigens.

V. A plan of smooth-rough form change which indicates these relationships was set up.

VI. Secondary cultures, created by addition of Vi-Bacteriophages to

strains containing Vi and O-Antigen, are not agglutinable and have a smooth colony form.

VI. Secondary cultures, created by addition of Vi-Bacteriophages to strains containing Vi and no O-Antigen, are autoagglutinable and have a rough colony form, even when the original strain was not only stable in table salt solution but also a smooth colony form.

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